Biology Undergraduate Research Symposium 2025

January 30th
12–4 pm

Hosted by Professor Adam Martin and Professor Laurie Boyer

SCHEDULE

12:00–12:50 PM

OPENING REMARKS

Lara Ozkan
Kellis Lab

Sofia Galiana
Case Lab

Isaac Lock
Page Lab

12:50 PM BREAK

1:00–1:50 PM

Sophia Cai
Soto-Feliciano Lab

Michaela Purvis
Martin Lab

Diya Ramesh
Vander Heiden Lab

1:50 PM BREAK

2:00–2:50 PM

Andrew Van Dusen
Yamashita Lab/Li Lab

Larissa Ma
Liu Lab

Esme Sun
Weissman Lab

2:50 PM BREAK

3:00–4:00 PM

Nupur Ballal
Prescott Lab

Jose Angel Cazares Torres
Uhler Lab

Victory Yinka-Banjo
Uhler Lab

CLOSING REMARKS

Contents

Sex-Specific Differences in Alzheimer's Disease

Lara Ozkan, Shanshan Zhang, Manolis Kellis

MIT Computer Science & Artificial Intelligence Laboratory, Cambridge, MA

Alzheimer's Disease (AD) is a progressive neurodegenerative disorder that significantly affects memory, behavior, and cognition, posing a growing public health challenge. With the rapid growth of aging populations, there is a critical need for research that incorporates diverse biological factors to develop more precise therapeutic strategies. Emerging evidence suggests that AD's pathophysiology has sex-specific variations due to genetic, molecular, and metabolic factors, which highlights the importance of tailoring interventions accordingly. This study integrates transcriptional, epigenomic, and metabolomic data to investigate how sex-specific factors influence AD incidence. We analyzed single-nucleus RNA sequencing (snRNA-seq), single-nucleus ATAC sequencing (snATAC-seq), and metabolite data from over 400 human post-mortem brain samples across multiple regions. By leveraging genome-wide association studies (GWAS) and functional quantitative trait loci (QTL) analyses, we uncovered sex-specific regulatory elements and metabolic pathways associated with AD. Preliminary findings suggest that genetic variants and metabolic profiles differ significantly between males and females. These differences may contribute to variations in disease progression, resilience, and therapeutic response. Metabolite data revealed additional pathways potentially implicated in sex-specific AD dynamics, offering a more comprehensive view of the interplay between genetic, epigenomic, and metabolic factors. This work highlights the importance of incorporating sex as a biological variable in AD research to uncover novel mechanisms and guide the development of personalized therapies. Future directions include conducting functional validation experiments to further understand the molecular drivers of these sex-specific differences and promote more equitable healthcare solutions for individuals affected by AD.

Faculty Supervisor: Manolis Kellis

Postdoc Mentor: Shanshan Zhang

Exploring the Role of Calpain Proteases in the Disassembly of Talin Condensates

Sofia Galiana, Kyle White, Lindsay Case

Dept. of Biology, MIT, Cambridge, MA

Focal adhesions are large protein assemblies that allow cells to attach to the extracellular matrix and are vital for cell migration during embryonic development, wound healing, and the immune response. Misregulation of focal adhesions also contributes to the metastasis and invasion of cancer cells highlighting their clinical and biological significance in disease progression. While the assembly of focal adhesions has been extensively studied, the disassembly of these complexes has not been as well-characterized or understood. Liquid-liquid phase separation is one of the driving forces for the formation of these focal adhesions, enabling dynamic assembly. Thus, focal adhesions are an example of a biomolecular condensate: cellular compartments that form through phase separation and are driven by interactions between macromolecules. Talin plays an important role in the focal adhesion complex, acting as a scaffold to link together various other proteins in the complex. We and others recently showed that purified talin undergoes phase separation in its open, uninhibited conformation, forming micron-sized liquid-like condensates *in vitro*. Calpain2, a calcium-dependent protease, has previously been shown to cleave talin at specific residues, and calpain2 protease activity is required for efficient focal adhesion disassembly in cells. Thus, we sought to test the effect of calpain2 on talin condensates. Here we show that calpain can disassemble preformed talin condensates. This implies that calpain2-mediated cleavage of talin specifically disrupts talin phase separation. Our results suggest that calpain2 protease activity is sufficient to disassemble talin condensates. Our study improves our understanding of the mechanism of cell migration by addressing the question of how calpain2 promotes focal adhesion disassembly.

Faculty Supervisor: Dr. Lindsay Case

Graduate Student Mentor: Kyle White

Investigating Dosage Sensitivity of ZFX and ZFY Using CRISPRi

Isaac Lock, Will Barr, David Page

Whitehead Institute, Cambridge, MA

The emergence of the mammalian XY sex-determination system came with the rapid loss of genes from the Y chromosome, and dosage compensation via X chromosome inactivation. However, there remain several genes which survive on the Y chromosome with homologs on the X that are not subject to inactivation. Many of these X-Y gene pairs are broadly expressed and encode regulators of gene expression, translation, and protein stability. Survival of these genes' Y homologs, along with their relative intolerance to mutation, suggests a high degree of dosage sensitivity. To investigate the tolerable range of expression of these genes, we utilized a CRISPR interference (CRISPRi) approach which incorporates mismatched sgRNAs to reduce knockdown efficiency to generate an allelic dosage series of ZFX and ZFY, an X-Y gene pair known to regulate the transcription of thousands of genes. We investigated the transcriptome-wide effects of precise variations in expression of ZFX and ZFY by performing bulk RNAseq on primary human fibroblasts from both male and female donors. We examined transcriptomic effects via differential expression analysis and discovered that transcriptome-wide effects are achieved with partial knockdown of ZFX and ZFY, providing further evidence for these genes' dosage sensitivity. To examine whether this degree of dosage sensitivity is evolutionarily conserved, we've developed a CRISRPi system in female chicken cells, which have a ZW sex chromosome complement. Chicken ZFX, the ortholog of human ZFX and ZFY, is not sex chromosomally encoded but present instead on chicken chromosome 1. Investigating genes with similar functions, but in different evolutionary lineages will hopefully grant useful insight into the evolutionary processes that led to the conservation of X-Y gene pairs in humans.

Faculty Supervisor: David Page

Graduate Student Mentor: Will Barr

Investigating the Tumor Suppressor Function of UTX Short Isoforms

Sophia Cai, Alireza Khademi, Yadira Soto-Feliciano

Koch Institute for Integrative Cancer Research, MIT, Cambridge, MA

Chromatin is the physiological form of the eukaryotic genome in which DNA is wrapped around histone proteins, allowing for the regulation of gene expression through histone modifications. Scaffold proteins are a class of chromatin regulatory proteins that regulate gene expression through non-catalytic means, namely through the recruitment of larger epigenetic complexes onto chromatin. UTX is a chromatin regulatory protein that possesses both a scaffolding and catalytic demethylase domain, yet studies have shown that it regulates development and functions as a tumor suppressor in various cancers independent of its catalytic function. Recent advancements have demonstrated that leukemia cells express a truncated, catalytically dead isoform of UTX that retains tumor suppressive function. In bladder urothelial carcinoma, UTX Q555* is a mutational hotspot that, if expressed, generates a similarly sized catalytic-dead isoform, $UTX_{1\text{-}554}$. To examine the tumor suppressive capabilities of UTX short isoforms, we investigated the effect of $UTX_{1\text{-}554}$ on *in vivo* tumorigenicity. We find that expression of $UTX_{1\text{-}554}$ confers tumor suppression in both immunocompetent and immunodeficient mice. In particular, tumor suppression is stronger in immunocompetent mice, suggesting an interplay between $UTX_{1\text{-}554}$ and the immune system. Additionally, by examining differential interactions between UTX isoforms through IP-MS, we show that while truncated UTX retains interactions with the canonical MLL3/4 complex, $UTX_{1\text{-}554}$ has unique interactions with antigen presentation machinery. Ultimately, we hypothesize that Q555* results in nonsense-mediated decay (NMD) of UTX mRNA, leading to the lack of functional UTX protein. Accordingly, restoration of a short UTX isoform can confer tumor suppression through both the canonical MLL3/4 complex and an enhanced immune response. Therefore, by characterizing the mechanisms by which truncated UTX confers tumor suppression, we aim to leverage truncated UTX as a novel therapeutic avenue through NMD inhibition or mRNA or protein delivery to treat cancers driven by UTX loss-of-function mutations.

Faculty Supervisor: Yadira Soto-Feliciano

Graduate Student Mentor: Alireza Khademi

Studying the Functions of Cytosolic Calcium Signals During *Drosophila* Gastrulation

Michaela Purvis, Juana De La O, Adam C. Martin

Dept. of Biology, MIT, Cambridge, MA

In embryonic development, cell signaling events are key to tissue formation and the genesis of three-dimensional form. The calcium ion is a versatile and dynamic signal throughout morphogenesis: it binds to certain cell adhesion molecules like cadherins, interacts with the actomyosin cytoskeleton, and activates actomyosin regulators such as gelsolin and calmodulin. Intracellular transporters and channels control the flow of calcium between the secretory pathway and the cytosol, allowing the cell to transiently increase cytosolic calcium, a phenomenon we call “calcium flashing”. Calcium flashes have been observed in a variety of morphogenic contexts for different purposes, including in the *Drosophila* embryo during tissue remodeling events. This is an ideal model to study these dynamics due to simpler yet well-conserved morphogenetic pathways and the ease of live imaging. Here, we use live *in situ* confocal imaging of embryos expressing a genetically encoded calcium indicator, GCaMP, to demonstrate that cytosolic calcium levels sporadically concentrate in single or groups of epithelial cells during germband extension. This actively displays calcium dynamics and potential signal patterns, though the function of calcium flashes for cell or tissue behavior is unclear. Furthermore, they appear to be heterogeneous across all observed embryos. Our current work focuses on characterizing flashes through imaging to categorize behaviors and correlated phenomena. While the functions for these flashes remains unknown, given their occurrence in the epithelium during germband extension, it is possible that they are associated with changes in cell shape or drive movement. A better understanding of the origin and types of flashes that occur during *Drosophila* gastrulation will provide greater insight into the diverse roles of calcium signaling in morphogenesis.

Faculty Supervisor and Mentor: Adam Martin

Cancer Cells Exhibit Increased Dependency on Methionine Synthase (MTR) When Exposed to Low-Lipid Environments

Diya Ramesh, Keene Abbott, Matthew Vander Heiden

Dept. of Biology, MIT, Cambridge, MA

Cancer cell metabolism is significantly influenced by microenvironmental factors such as nutrient availability, which can vary across different tissues and introduce exploitable therapeutic vulnerabilities[1]. Notably, lipid availability is particularly low in the brain compared to other tissues[2]. To identify metabolic vulnerabilities of cells in lipid-poor environments, we performed a CRISPR/Cas9 screen of metabolic synthesis genes in human cancer cells cultured in lipid-rich versus lipid-poor media. Through this screen, we observed an increased dependency on the folate metabolism gene methionine synthase (MTR) in the cells when grown in lipid-depleted conditions. We verified this differential dependency by knockout (KO) of MTR, which resulted in slower cell proliferation in lipid-depleted media. Additionally, wild-type cells displayed heightened sensitivity to inhibitors of other proteins in the pathway in lipid-depleted conditions, thereby phenocopying the MTR KO. Polar metabolomics analyses by LC-MS revealed depletion of several nucleotide species in the MTR KO cells cultured in lipid-depleted conditions, and supplementation with purine nucleotides rescued the proliferation of MTR KO cells cultured in lipid-depleted media. We hypothesized that this nucleotide depletion could in turn result in nucleotide imbalance and DNA damage, and Western Blot analyses confirmed elevated DNA damage levels in the MTR KO cells in lipid-depleted media. Our model is that in low-lipid environments, the MTR KO redirects metabolic resources toward lipid synthesis, causing nucleotide imbalance, DNA damage, and cell death. Overall, our findings indicate that cancer cells rely more on MTR and folate metabolism when exposed to lipid-depleted environments, presenting a novel synthetic lethality that could be leveraged for treating tumors growing in lipid-poor organs such as the brain.

1 Muir A and Vander Heiden MG (2018) Science
2 Ferraro et al. (2021) Nature Cancer

Faculty Supervisor: Matthew Vander Heiden

Graduate Student Mentor: Keene Abbott

The Function of Ovo in Regulating Transposable Elements in the *Drosophila* Male Germline

Andrew Van Dusen, Amelie Raz, Yukiko Yamashita

Whitehead Institute, MIT, Cambridge, MA

The transcription factor Ovo is well-known for its essential role in the development of the female germline in animals ranging from flies to humans. In contrast, it has been thought that Ovo serves no function in the male germline. However, utilizing RNA fluorescence *in situ* hybridization and immunofluorescence, we found that in *Drosophila* male germline stem cells (GSCs), Ovo is indeed necessary for the expression of Piwi, a piRNA-binding protein responsible for transcriptionally silencing transposable elements. In *Drosophila* males, when rDNA copy number is low, the GSCs will derepress the R2 retrotransposon in order to generate double-stranded breaks at the rDNA locus, allowing for unequal sister chromatid exchange and a preferential segregation of the chromatid which gained rDNA to the GSC. Interestingly, we found using both RNA FISH and qPCR that both Ovo isoforms are significantly upregulated under low rDNA conditions. We also observed that in flies with the ΔOvoB mutation, a truncated form of R2 – which arises from incomplete reverse transcription and results in non-functional ribosomes – becomes derepressed. However, in flies only possessing low rDNA copy number, there is no derepression of truncated R2, despite there being derepression of full-length R2. This revealed to us that when R2 is derepressed to increase germline rDNA copy number, Ovo may be upregulated in order to increase expression of Piwi and therefore silence harmful truncated R2 copies. This also aligns with an observation that the piRNAs against R2 are all directed against its 3' end, which would therefore preferentially silence truncated R2. We are now interested in seeing how the expression of full-length and truncated R2 are affected in flies that have both the ΔOvoB mutation and low rDNA copy number, hopefully better informing us about transposable element regulation in the germline.

Postdoc Mentor: Amelie Raz

Faculty Supervisor: Yukiko Yamashita

Developing a Tool for Targeted Enrichment and Spatial Mapping of Lowly Expressed Transcripts

Larissa Ma, Ally Vernich, Kevin Chen, Sophia Liu

Ragon Institute, 600 Main St, Cambridge, MA

B cells play an indispensable role in the adaptive immune response, mediating antigen recognition and antibody production through diverse B-cell receptors (BCRs) generated by somatic hypermutation. Human immunodeficiency virus (HIV) attacks and destroys CD4+ T cells, weakening the immune system and evading immune recognition through its high mutation rate. Determining the spatial distributions of immune cells and interactions with pathogens is essential to understanding the adaptive immune response. Existing methods to spatially profile BCRs and HIV in tissues are often labor intensive, targeted/low-throughput, or lack single-cell resolution. In particular, BCR and HIV transcripts are long and lowly expressed in tissue, making them difficult to capture. To address these challenges, we combined Slide-seq, a bead-based spatial transcriptomics technology, with a targeted enrichment protocol to enhance the study of lowly expressed transcripts such as BCRs and HIV. We optimized a hybridization-based capture method that integrates isothermal amplification, reverse transcription, and pulldown of single-stranded DNA using biotinylated probes to enrich specific transcripts from cDNA libraries. For studying HIV, specific primer sequences were designed to target consensus regions across all HIV subtypes, ensuring efficient capture despite sequence variability. This method achieves sensitivity and specificity comparable to existing spatial profiling technologies while overcoming challenges with low transcript expression. Future research will study how the immune response is spatially organized in the site of first infection in human HIV samples, providing insights into the spatial and temporal dynamics of HIV and immune cells.

Faculty Supervisor: Alison Ringel

Mentor: Sophia Liu

Development of a Programmable HUSH Complex Recruitment Tool

Elizabeth M. Sun, Victory Yinka-Banjo, Tessa M. Bertozzi, Jonathan S. Weissman

Dept. of Biology, Whitehead Institute, MIT, Cambridge, MA

The human silencing hub (HUSH) complex is a key epigenetic repressor that protects the human genome by blocking transcription of retroelements, RNA-derived sequences integrated into the host genome. The HUSH complex targets both foreign retroviruses and endogenous retrotransposons, such as LINE-1 elements and integrated HIV. It is composed of three core protein subunits: transcription activation suppressor (TASOR), M-phase phosphoprotein 8 (MPP8), and periphilin (PPHLN). To mediate transcriptional repression, the HUSH complex recruits the histone methyltransferase SETDB1 and the chromatin remodeler MORC2.

The HUSH complex distinguishes host genes from retroelements by recognizing and repressing long, intronless transcripts. However, its targeting and recruitment mechanisms remain poorly understood.[1] To address this knowledge gap, we developed a programmable CRISPR-based epigenetic silencing tool to artificially recruit the HUSH complex to a desired gene locus. Specifically, we fused periphilin to the RNA-targeting catalytically inactive Cas13 protein (dCas13) and showed that artificially recruiting periphilin to nascent RNA is sufficient to silence a human reporter gene in cultured human cells. We confirmed that the silencing was HUSH-mediated by assessing the silencing ability of TASOR-binding loss-of-function point mutants and periphilin truncations. Our optimized periphilin-dCas13 tool can achieve robust silencing (~90%) of our reporter in human HEK293T cells.

To elucidate the targeting rules of our periphilin-dCas13 tool, we conducted a screen of 3,000 single guide RNAs (sgRNAs) tiling the nascent gene transcript. We found that effective silencing requires sgRNAs targeting early regions within the first intron. Beyond enabling the mechanistic dissection of HUSH silencing, our novel periphilin-dCas13 tool expands the repertoire of transcriptional repression technologies and offers potential for targeting genes resistant to current silencing methods.

1 Seczynska, M., Bloor, S., Cuesta, S.M. et al. (2022) Nature. 601, 440–445

Faculty Supervisor: Jonathan S. Weissman

Postdoc Mentor: Tessa M. Bertozzi

Characterizing Airway Intrinsic Neurons with Viral Sparse Labeling Techniques

Nupur Ballal, Julia J. Van Goor, Sara L. Prescott

Dept. of Biology, MIT, Cambridge, MA

Neurons are essential for coordinating breathing and protecting the airways from various threats. Airway-intrinsic neurons (AINs) are one of many sources of airway innervation and reside fully within airway tissue, forming an interconnected network. They are broadly believed to regulate mucus secretion, bronchoconstriction/dilation, vasoconstriction/dilation, and immune activation. Historically, these neurons have been understudied due to their relative rarity and location, but recent technological developments have enabled them to be studied with molecular resolution. The Prescott lab has generated the first unbiased profile of the molecular diversity of airway-resident neurons using single cell RNA sequencing, however these data lack spatial information. Given that neuron morphology has historically been used to define cellular subtypes, mapping the cellular architecture of this network will complement the sequencing data and help generate hypotheses about potential functions of AIN subtypes. Since the airways receive innervation from multiple sources, the density of axons and dendrites necessitates the use of new sparse labeling strategies to target only the neuronal fibers originating from AINs. Thus, we piloted the use of adeno-associated virus (AAV) to deliver genetic cassettes containing Cre- dependent fluorescent proteins to various mouse lines expressing Cre recombinase in different subpopulations of AINs, as informed by single cell sequencing data. As different viral serotypes infect cell types with varying efficiency, we tested serotypes AAV9 and AAV-PHP.S, which have already been shown to efficiently label neurons in peripheral organs. Using a Cre- and dose-dependent mechanism, the AAVs sparsely labeled AINs with a fluorescent protein, and were co-immunostained with other antibodies to label additional cell types. Sparse labeling of AINs will provide critical information regarding the spatial organization and innervation targets of these airway-resident neurons, which may help inform future druggable targets for airway diseases in which dysregulated neuronal signaling has been implicated, like COPD and asthma.

Faculty Supervisor: Sara L. Prescott

Graduate Student Mentor: Julia J. Van Goor

Optimal Transport for Perturbation Distances

Jose Angel Cazares Torres, Jiaqi Zhang, Pinar Demetci, Caroline Uhler

Eric and Wendy Schmidt Center, MIT, Cambridge, MA

Understanding the effects of genetic perturbations is crucial for uncovering gene regulatory mechanisms and identifying context-specific molecular pathways. Perturb-seq, a CRISPR-based high-throughput technique, enables transcriptomic profiling of single cells that underwent gene perturbations, offering an opportunity to study perturbational effects at scale. This project aims to formally quantify the (a)similarities between different perturbations using genome-wide Perturb-seq data1 by mapping transcriptomic readouts to a space where the geometry reflects such (a)similarities. Because of the high-dimensional, stochastic, and sparse nature of single-cell data, defining robust and informative similarity measures poses a challenge. To address this, we leverage optimal transport (OT), a mathematical framework that provides geometrically meaningful distances between probability distributions. OT computes distances between gene expression profiles by comparing pairs of genes, and it can flexibly incorporate prior information on gene-gene relationships—e.g., pathway information—as cost matrices. We aim to learn cost matrices between genes in a way that is consistent with the perturbational effects of targeting genes using coupled optimal transport, for instance, two genes with similar perturbational effects should correspond to a lower cost, and vice versa. Such an approach holds promise for obtaining a biologically meaningful manifold of genes, which we observe in our initial results when compared to traditional Euclidean manifolds.

1 Replogle, J.M, et al. "Mapping Information-Rich Genotype-Phenotype Landscapes with Genome-Scale Perturb-Seq." Cell 185, no. 14 (July 2022). https://doi.org/10.1016/j.cell.2022.05.013.

Faculty Supervisor: Caroline Uhler

Post-Doctoral Mentor: Pinar Demetci

Graduate Student Mentor: Jiaqi Zhang

Representation Learning Associates Patients' Risk for Metabolic Disease with Their Image-based Lipocyte Cell States

Victory Yinka-Banjo, Xinyi Zhang, Zipei Tan, Carol Chen, Hesam Dashti, Felipe dos Santos, Melina Claussnitzer, Caroline Uhler

Department of EECS, MIT, Eric and Wendy Schmidt Center, Broad Institute of MIT and Harvard, Cambridge, MA

Polygenic risk scores (PRS) estimate an individual's genetic risk of developing a certain disease, suggesting that differences between cells of individuals with high versus low PRS could give us insight into the cellular disease states. To study metabolic diseases, we associate individual-level genotypes with cell-level features. We accomplish this by making use of a recent large-scale lipocyte (fat cells) microscopy imaging dataset. By learning a representation of multi-channel lipocyte microscopy images using a convolutional variational autoencoder, we performed unsupervised clustering on the learnt representations to identify different cell states. We then analyzed the distribution of these cell states in different individuals and associated their PRS to the observed cell state distributions. We also identified PRS that differentiate cells treated with free fatty acid (associated with metabolic disease) from untreated cell populations. Finally, we show that it is possible to generate counterfactual lipocyte images and understand the effect of increased or reduced PRS on metabolic disease cell states through transforming the learnt representations. These results demonstrate that associating genotypic individual-level PRS and cell-level microscopy images can offer a promising approach to characterize metabolic disease while leveraging low-cost imaging techniques for more accessible diagnostics.

Faculty Supervisor: Caroline Uhler

Graduate Student Mentor: Xinyi Zhang

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